Insulin resistance and IGF-I sensitivity in vascular cells
- impact of hybrid receptors

With special regard to diabetes

Git Johansson
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Cover: the hybrid insulin receptor / insulin-like growth factor receptor

During the course of the research underlying this thesis, Git Johansson was enrolled in Forum Scientium, a multidisciplinary doctoral programme at Linköping University, Sweden.

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Skam den som ger sig...
Insulin resistance and IGF-I sensitivity in vascular cells
- impact of hybrid receptors

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ABSTRACT

Diabetic complications largely affect the circulation and are associated with resistance to insulin and altered levels of insulin-like growth factor-I (IGF-I). Insulin resistance and altered IGF-I levels are also associated with vascular disease. Insulin and IGF-I are highly homologous peptides and can cross react with each others respective receptors, insulin receptors (IR) and IGF-I receptors (IGF-IR), which also share homology to a large extent and can form hybrid IR/IGF-IR. Cultured endothelial and vascular smooth muscle cells from different vascular beds express considerably more IGF-IR than IR. Since the direct action of insulin and IGFs on the vasculature remains poorly understood, our aim was to study mechanisms behind insulin resistance and IGF-I sensitivity and the possible impact of hybrid IR/IGF-IR in vascular cells.

This thesis is based on four papers investigating the presence of IR and IGF-IR in cultured endothelial and vascular smooth muscle cells, and in tissue specimens from human left internal mammary artery (LIMA). We examined, in cultured vascular smooth muscle cells and endothelial cells, the phosphorylation of IR and IGF-IR, and IR and IGF-IR mediated actions, i.e., subsequent downstream signalling and biological effects, in response to physiologic and supraphysiologic concentrations of insulin, IGF-I and IGF-II. We also examined the presence of insulin/IGF-I hybrid receptors in these cell types. To compare our results in vitro with the in vivo situation we investigated the relative gene expression of IGF-IR to IR in LIMA.

We conclude that: 1) the relative abundance of IGF-IR is considerably higher than IR in vascular cells in vitro and in vivo; 2) in addition to IR and IGF-IR, hybrid IR/IGF-IR are present in vascular cells; 3) IR activation at physiological concentrations (≤10⁻⁹M) does not propagate downstream signalling and biological effects in endothelial and vascular smooth muscle cells; 4) low concentrations of IGF-I activate IGF-IR, as well as IR due to the presence of hybrid IR/IGF-IR, and propagate downstream signalling and biological effects in endothelial and vascular smooth muscle cells; and 5) the biological effects mediated by IGF-II suggests a role for IGF-II in vascular smooth muscle cells.

The papers included in this thesis provide new insight on how IGFs and insulin act in the vasculature. The preponderance of IGF-IR relative to IR in addition to sequestration of IR into hybrid IR/IGF-IR contributes to an insulin resistance located at the receptor level in endothelial and vascular smooth muscle cells. Hence, our results suggest that IGFs rather than insulin have an impact on vascular function.
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ABBREVIATIONS

Akt  protein kinase B
BP  binding protein
FFA  free fatty acids
EC  endothelial cells
eNOS  endothelial nitric oxide synthase
Erk 1/2  extracellular signal-regulated kinases 1 and 2
GAPDH  glyceraldehyde-3-phosphate dehydrogenase
GH  growth hormone
GLUT  glucose transporter
HASMC  human aortic smooth muscle cells
HMVEC  human microvascular endothelial cells
HUVEC  human umbilical cord vein endothelial cells
Hybrid IR/IGF-IR  hybrid insulin receptor/insulin-like growth factor-I receptor
IGF  insulin-like growth factor
IGF-I  insulin-like growth factor-I
IGF-IR  insulin-like growth factor-I receptor
IGF-II  insulin-like growth factor-II
IGF-IIR  insulin-like growth factor-II receptor
IGFBP  insulin-like growth factor binding protein
IP  immunoprecipitation
IR  insulin receptor
IR-A  insulin receptor isoform A
IR-B  insulin receptor isoform B
IRS  insulin receptor substrate
LIMA  left internal mammary artery
MAPK  mitogen-activated protein kinase
mRNA  messenger ribonucleic acid
NO  nitric oxide
PDK1  phosphoinositide-dependent kinase 1
PI-3K  phosphatidylinositol-3 kinase
PIP3  phosphatidylinositol-3, 4, 5-triphosphate
PKB  protein kinase B, usually called Akt
PKC  protein kinase C
RT-PCR  reverse transcription polymerase chain reaction
SDS-PAGE  sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SOS  son-of-sevenless
VSMC  vascular smooth muscle cells
WB  Western blot
LIST OF PAPERS

Paper I  **Git S Johansson** and Hans J Arnqvist
Insulin and IGF-I action on insulin receptors, IGF-I receptors, and hybrid insulin/IGF-I receptors in vascular smooth muscle cells

Paper II  **Git S Johansson**, Simona I Chisalita and Hans J Arnqvist
Human microvascular endothelial cells are sensitive to IGF-I but resistant to insulin at the receptor level.
Molecular and Cellular Endocrinology. 2008; **296**: 58-63.

Paper III  Simona I Chisalita, **Git S Johansson**, Ellinor Liefvendahl, Karolina Bäck and Hans J Arnqvist
Human aortic smooth muscle cells are insensitive to insulin at the receptor level but sensitive to IGF-I and IGF-II.
Submitted

Paper IV  **Git S Johansson**, Rolf Svedjeholm and Hans J Arnqvist
High expression of IGF-I receptors compared to insulin receptors in human mammary artery.
*In manuscript*
INTRODUCTION

Cardiovascular disease is the major cause of morbidity and mortality in Western societies. Diabetes type 1 and type 2, as well as insulin resistance are prominent risk factors for cardiovascular complications (1, 2) and diabetes is associated with specific lesions in the microcirculation, diabetic microangiopathy (3). Diabetes is due to insufficient insulin secretion which causes hyperglycaemia, but this also has consequences for the insulin-like growth factor (IGF)-system and circulating insulin-like growth factor type I (IGF-I) activity (4). Alterations in circulating IGF-I activity are also associated with vascular disorders (5-7). The reason for the increased risk for cardiovascular disease in these conditions is less well understood. The focus of this thesis has been to study the presence and impact of IGF-I receptor (IGF-IR), insulin receptor (IR) and hybrid IR/IGF-IR on insulin resistance and IGF-I sensitivity in vascular cells.

The insulin-like growth factor (IGF) system

The IGF system (Figure 1) is an evolutionarily conserved system involved in regulation of growth, proliferation and survival. It includes 1) the polypeptides; IGF-I, IGF-II and insulin, 2) the IGF binding proteins-1-6, 3) the receptors; IGF-IR, IR, hybrid IR/IGF-IR, IGF-IIR; and 4) IGFBP-proteases. (as reviewed in 8 and 9) IGF-I, IGF-II and insulin are highly homologous peptides, derived from a common ancestral precursor hormone (10). IGF-I and IGF-II are single chain polypeptides with 70 and 67 amino acids, respectively. The IGF-I and IGF-II molecules are arranged in 4 domains where the A and B domains share amino acid sequence homology with insulin, whereas C and D are unique for IGF-I and IGF-II (Figure 2) (reviewed in 11).

Insulin-like growth factor type I (IGF-I)

IGF-I is predominantly synthesised by the liver, but is also produced locally in most tissues where it acts in a paracrine/autocrine manner. IGF-I has endocrine effects in many target tissues and a number of important functions in the body such as stimulation of amino acid uptake and protein synthesis, promotion of cell migration, regulation of cell cycle progression and mitogenesis, inducing cell cycle changes and also protecting from apoptosis thus opposing programmed cell death (reviewed in 12). IGF-I has also been suggested to have a role in glucose and lipid metabolism, promoting peripheral uptake of glucose and protect against insulin resistance (reviewed in 8). It may also be involved in blood pressure regulation via changes in peripheral resistance by increase in cardiac output and stroke volume (13; 14).
The insulin growth factor (IGF) system

Fig 1. The insulin growth factor (IGF) system including polypeptides, receptors and IGF binding proteins (IGFBPs).


Fig 2. IGF-I and insulin
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**Insulin-like growth factor type II (IGF-II)**
IGF-II is a close relative to IGF-I and is thought to have its main effect during pre-natal life, being important for normal foetal growth and development, as seen in both murine models and humans (15). In the human circulation IGF-II is found throughout life and is actually the predominant IGF in adult humans, the levels being 5-10-fold higher than IGF-I (16). IGF-II propagates its effects primarily through the IGF-IR, to which it can bind with as high affinity as to its own receptor IGF-IIR, and also through the IR isoform A to which it binds with equal affinity as insulin (17).

**Insulin-like growth factor type II receptor (IGF-IIR)**
The IGF-IIR, also the receptor for mannose-6-phosphate and called mannose-6-phosphate-R, binds IGF-II with high affinity and was thought until recently only to serve to limit IGF-II bioavailability by targeting IGF-II for degradation (15). However, studies on endothelial progenitor cells from umbilical cord blood showed endothelial progenitor cells to have a high expression of IGF-IIR, where IGF-II and IGF-IIR seem to contribute to vasculogenesis at ischaemic or tumour sites (18). Furthermore, the IGF-IIR might have role in tumour suppression (19).

**Insulin-like growth factor binding proteins (IGFBPs)**
Free IGFs in the circulation is regulated by IGF-binding proteins (IGFBP) 1-6, mainly IGFBP-3, acting as IGF storage in the blood. In vivo, less than 1% of IGF-I and IGF-II is free and bioactive, the rest is bound to IGFBPs (16). IGFBPs control IGF bioavailability by forming complexes with IGFs, thereby prolong their metabolic half-life and modulate the direct binding of IGFs to their receptors. IGFBP-3 is the most common IGFBP and forms a ternary complex together with IGF and an acid-labile subunit. The IGF-I-IGFBP-3 complex extends the IGF-I half-life in serum from 10 minutes to 12-15 hours by preventing IGF-I proteolysis. IGFBP-1 may regulate glucose levels by inhibiting IGF-I action. (as reviewed in 8, 14)

**Insulin**
Insulin is the primary regulator of glucose metabolism by regulating glucose uptake and glucose production, and other metabolic actions as lipolysis and protein synthesis. Insulin is secreted from β-cells in the pancreas in response to high blood glucose and amino acids. In the circulation insulin is free, ie not bound to binding proteins, and thereby able to act quickly. Unlike IGFs, insulin concentration changes rapidly during the day, with high postprandial peaks in response to meals, and low levels between meals and during the night (16).
**Insulin analogues**

Insulin therapy is needed to maintain normoglycaemia in patients with type 1 diabetes, and is also needed in many patients with type 2 diabetes. A major problem in the therapy of insulin-deficient patients has been to obtain the rapid postprandial rise in plasma insulin that normally occurs in the nondiabetic individual. On the other hand, during the night and between the meals a long-acting insulin is needed for substitution of basal insulin requirement.

The Asp B10 analogue was the first insulin analogue developed for clinical use through recombinant gene technology. In experiments on cultured cells, however, it was shown to have a 10-20 fold increased mitogenic activity, probably due to it’s increased affinity for IGF-IR, and it was also found to produce mammary tumours in rats (20, 21). Subsequently developed rapid-acting insulin analogues, lispro, aspart and glulisine have no increased mitogenic activity and no increased affinity for the IGF-I receptor and they are now widely used in diabetes care (22; 23). The first long-acting insulin analogue was glargine, which has the same affinity for the IR as human insulin but a 5-10 fold increased affinity for the IGF-I receptor (24; 25). This has been a matter of concern but has probably no clinical relevance at therapeutic concentrations (25; 26).

**Insulin receptor and IGF-I receptor**

The insulin receptor (IR) and IGF-I receptor (IGF-IR) are homologues having 84% homology in the β-subunit tyrosine kinase domains. Both belong to the tyrosine kinase family. Unlike other receptor tyrosine kinases, which are activated by ligand dimerisation, IR and IGF-IR exist at the cell surface already dimerised as a tetramer comprised of two disulphide-linked αβ-dimers, where each αβ-dimer consists of an extracellular α-subunit and a transmembrane β-subunit (27, 28). The extracellular portions of the IR and IGF-IR, the α-subunits, contains six structural domains, of which ligand-binding determinants have been localised to the L1, cysteine-rich and the L2 domains in the N-terminal half, and in the C-terminal half peptide sequence (29). The intracellular portions of the receptor, the β-subunits, are the parts of the receptor actually being tyrosine phosphorylated and subsequently transduce the signal further into the cell.

**Regulation of the IGF system**

Circulating IGF-I and IGF-II are bound to IGFBPs, mainly IGFBP-3, which serves as a storage for IGF in the circulation, only a small fraction of IGFs are free and bioactive. The production of IGF-I is regulated by growth hormone (GH) secretion and probably also by the expression of GH-receptors in the liver (16). During a lifetime GH-secretion changes, reaching a peak at puberty then declining during adult life. This is reflected in a similar profile for circulating IGF-I (30).
Under normal conditions insulin is secreted into the hepatic portal vein and reaches the liver in a concentration several fold higher than in peripheral blood (31). In the liver insulin can influence IGF-I bioavailability in two ways, by up-regulating or down-regulating the GH-receptors thereby making the liver GH-sensitive or insensitive (16), and by acting on IGFBP-1. IGFBP-1 is transcriptionally regulated by insulin in the liver and changes within hours. High levels of insulin down-regulates IGFBP-1 and thereby increases IGF-I bioavailability (32). As depicted in Figure 3, in diabetes, insulin release into the portal vein is abolished and insulin delivery to the liver will therefore be much reduced (marked with an X). It has been shown that in spite of normal or near normal glycaemic control acquired with the help of insulin therapy, in patients with type 1 diabetes lacking endogenous insulin secretion, there are alterations in the IGF-system with low free IGF-I and high IGFBP-1 levels (33). This is probably because insulin administered in peripheral blood will not result in the high concentration of insulin in the hepatic portal vein needed to influence IGF-I bioavailability. Fasting also affects the IGF-system probably by reducing circulating insulin and leads to reduction in IGF-I levels. IGF-I infusion in the fasting state somehow enhances insulin sensitivity by enhancing insulin action in peripheral tissues (34).

**Hybrid insulin receptor / IGF-I receptor**

In tissues co-expressing IGF-IR and IR, the receptors can heterodimerise to form hybrid receptors, hybrid IR/IGF-IR consisting of one IR αβ-dimer and one IGF-IR αβ-dimer (35, 36). Hybrid receptors have been demonstrated in many tissues including skeletal muscle, adipose tissue, placenta, breast cancer cells and osteoblasts. (reviewed in 37, 38). The hybrid IR/IGF-IR has binding characteristics similar to the IGF-IR, and binds IGF-I, but not insulin, with high affinity (36). The formation of hybrid receptors is thought to be regulated by a process of random assembly determined by the molar proportion of receptors, i.e. the relative stoichiometric abundance of IGF-IR and IR determines the abundance of hybrid receptors. In effect, the less abundant receptor will, to a large extent, be incorporated into hybrid IR/IGF-IR (39).

The presence of hybrid receptors as a potential regulative mechanism within the IGF-system must be taken into consideration. Due to the process of random assembly upregulation or downregulation of either receptor will lead to an alteration of the proportion of hybrid IR/IGF-IR, and thereby regulate the peptides’ biological effects (40, 41, 42).
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Fig 3 Regulation of insulin growth factor (IGF) system

Fig 4 The IGF-I receptor (IGF-IR), insulin receptor (IR) and hybrid IR/IGF-IR
**Ligand binding**

The first step in the initiation of the biological effects of insulin, IGF-I and IGF-II, is binding to their membrane-bound cell surface receptors, IGF-IR, IR or hybrid IR/IGF-IR. Ligand binding is a consequence of cross-linking of α-subunits by the ligand which induces a conformational change resulting in ATP binding and autophosphorylation of the β-subunit.

The insulin receptor contains two binding sites consisting of two epitopes each, which are situated on the extracellular IR α-subunits. High-affinity binding of one insulin molecule requires binding of both binding sites, located one on each side of the molecule, to the pair of binding epitopes located one on each of the receptor α-subunits. For high-affinity binding of insulin to IR to occur, binding to both receptor α-subunits is necessary, whereas IGF-I needs only one IGF-IR α-subunit to bind with high affinity (43, 44). The need to bind only to one high-affinity binding site has been suggested to be case for IGF-II as well (45). Insulin binding to the insulin receptor exhibits negative cooperativity, i.e. insulin affinity for binding sites decreases with occupancy (46). Binding of IGF-I to the IGF-IR also exhibits negative cooperativity (47).

The fact that IGF-I needs only one IGF-IR α-subunit to bind with high affinity probably explains why insulin has a low affinity for insulin/IGF-I hybrid receptors, while IGF-I can bind with the same high affinity as to IGF-I homodimers. In the hybrid receptor the IR αβ-dimer can be cross-activated through activation of the IGF-IR αβ-dimer, i.e. the insulin receptor half can be activated as well (reviewed in 44).

**Activation of receptors and signalling pathways**

The conformational change of the receptor caused by ligand binding will bring the domains close together and lead to autophosphorylation of several tyrosine residues on the β-subunits (48). This is then followed by recruitment and phosphorylation of docking proteins including IRS proteins, Erk (extracellular signal-regulated kinase) 1 and 2 and Akt (also known as protein kinase B) involved in the regulation of cell metabolism, proliferation, and survival, depending on cell type, see Fig 5 (49, 50, 28). IGF-IR and IR share largely overlapping signalling pathways, the critical nodes being IRS (insulin receptor substrate) proteins 1-4, phosphatidylinositol 3-kinase (PI3K) and Akt in the PI3K-pathway responsible for most of the metabolic effects, and Erk 1/2 in the Ras-mitogen-activated protein kinase (MAPK) pathway which in cooperation with the PI3K-pathway controls mitogenic and differentiation responses (51).
IGF-IR IR signalling

Fig 5 Intracellular signalling of insulin

IRS proteins include IRS isoforms 1-4, of which IRS-1 and IRS-2 are widely distributed and thought to be involved in somatic growth and nutrient homeostasis. Activation of the receptors by IGF-I and insulin results in tyrosine phosphorylation of the IRS proteins which bind PI3K and the adaptor molecule Grb-2 which associates with son-of-sevenless (SOS). The Grb-2/SOS-complex further activates the MAPK pathway, including the Ras – Raf – MEK – Erk 1/2 cascade. (as reviewed in 51 and 52)

When activated, PI3K will catalyse the formation of the lipid 2nd messenger phosphatidylinositol-3, 4, 5-triphosphate (PIP3), which will phosphorylate the 3-phospho-inositide-dependent protein kinase 1 (PDK1), which in turn will phosphorylate Akt (reviewed in 53). Knockout studies in mice have shown Akt1 to be the predominant isoform in vascular cells (54). IGF-I acts antiapoptotic via the PI3K pathway, where the activation of PI3K is followed by Akt phosphorylation and subsequent phosphorylation and inactivation of the proapoptotic Bad, caspase 9 (55).
Insulin receptor isoforms

The human IR exists in two isoforms; IR-A, which lacks exon 11, encoding 12 amino acids at the C-terminal of the α-subunit, and IR-B which contains this sequence. Due to the exon 11 sequence the isoforms have been shown to have different binding affinities and responses, the IR-B has an affinity for insulin higher than that of IR-A, and mediating predominantly metabolic effects, whereas for IGF-II, IR-A is a high-affinity receptor for IGF-II, mediating predominantly proliferative response (17, 56-58). Either isoform has high-affinity for IGF-I, but IR-A has a higher binding affinity for IGF-I than IRB. The difference in binding affinities has drawn special attention since the isoforms have been shown to be differently expressed in diabetes patients compared to normal (57, 59, 60). Earlier studies have shown hybrid IR/IGF-IR to have different affinities depending on which IR isoform is involved (61), ie either hybrid IR-A/IGF-IR or hybrid IR-B/IGF-IR, but more recent studies suggest that the affinities are similar (29, 62). Also, using BRET technique, hybrid receptors consisting of one IR isoform each, ie hybrid IR-A/IR-B, has recently been shown to form in tissues co-expressing IR-A and IR-B (45). Their binding characteristics was shown to be somewhat divergent compared to homoreceptors, they bind IGF-I similar to IR-B and IGF-II similar to IR-A, but no difference was found in insulin affinity compared to IR homoreceptors.

Insulin and IGF-I in classical insulin-target tissues

The effect of hormones in various tissues depends on type of target cell together with receptor distribution, receptor type and receptor abundance. Although IR and IGF-IR have similar actions to some extent, one cannot compensate for a deficiency in the other’s function. In mice, both IGF-IR and IR null mutants die at birth or shortly thereafter, due to respiratory failure or metabolic complications respectively (63, 64). IGF-IR are widespread in the body and are important for growth in all organs. Regarding IR, however, the high concentration of IRs is concentrated to classical insulin-target tissues, including skeletal muscle, liver and fat. Insulin enables glucose uptake in these tissues by translocating the glucose transporter (GLUT) 4 to the cell membrane, as in muscle and fat, or by regulating intracellular enzymes, as in the liver. Insulin also directly suppresses glucose production by the liver.

The skeletal muscle is the principle site of insulin-stimulated glucose uptake, accounting for 75% of glucose disposal in the postprandial state, making it the main target for studies of insulin resistance. In addition to IRs, skeletal muscle has a large concentration of IGF-IR, and, when activated, they as well enhance muscle glucose uptake via mobilisation of GLUT-4. Inactivation of IGF-IR in skeletal muscle in mice causes severe insulin resistance and type 2 diabetes at an early age, implicating an important function for IGF-I in glucose homeostasis (65). This is in agreement
with a recent study on myoblasts and myotubules showing IGF-IR mRNA to be 5 times more abundant than IR. Furthermore IGF-I had a significant effect on microarray gene expression in myoblasts and myotubules whereas insulin did not (66).

**Insulin resistance and Type 2 Diabetes**

Insulin resistance is defined as a state of decreased responsiveness of insulin target tissues to normal circulating levels of insulin and is the major cause of Type 2 Diabetes, but is also a feature of a number of other health disorders, including obesity, glucose intolerance, dyslipidemia and hypertension, clustering in the so-called metabolic syndrome (reviewed in 67), and arterial stiffness (68). Initially, β-cells can increase in mass and relatively increase insulin secretion to compensate for the insulin-resistant state, but will eventually be exhausted and fail to respond to the impaired glucose disposal. The β-cell dysfunction together with the insulin resistance will cause hyperglycemia and diabetes (reviewed in 67). There are several mechanisms proposed to be involved in the pathophysiology of insulin resistance; the influence of increased levels of free fatty acids and pro-inflammatory molecules, impaired muscle glycogen synthesis, defects in glucose uptake and insulin intracellular signalling, and more (reviewed in 67). Interestingly, in classical insulin target tissues, the expression of hybrid IR/IGF-IR have been correlated with insulin sensitivity in vivo (69, 70).

**The vasculature – a non-classical insulin target tissue?**

Over the past decades, it has been increasingly recognised that vasculature, particularly the endothelium, might be an important physiological target for insulin. Clinical studies in vivo have demonstrated obvious circulatory effects of insulin, where insulin has been shown to relax conduit vessels in healthy, but not insulin resistant, probably by stimulating endothelial nitric oxide synthase (eNOS) to produce vasodilating nitric oxide (NO) (reviewed in 71). Also, the vasodilating effect of insulin in vivo on peripheral resistance arteries in skeletal muscle have been detected, however, it was slow and required supraphysiological concentrations of insulin (71). This suggests insulin might have been acting via the IGF-IR, or possibly hybrid IR/IGF-IR, which, as mentioned earlier, has been shown to be of importance for development of insulin resistance in skeletal muscle (65), there by implicating an important role of IGF-I and IGF-IRs in skeletal muscle vasculature.

Interestingly, regarding the connection between insulin resistance and vascular complications, the β-cell dysfunction has been proposed to be due to defective vascular function in the β-cells, ie that diabetes occurs as an effect of vascular complications instead of the other way around (72).
The vascular wall

In larger arteries, the vascular wall is comprised of three layers, the inner tunica intima (endothelial cells), the middle tunica media (smooth muscle cells) and the outer layer tunica adventitia (connective tissue). Microvessels consist of arterioles, capillaries and venules. These vessels differ from macrovessels with respect to architecture and cellular components. While larger vessels provide blood to organs, microvessels have specific roles regulating blood pressure and delivering nutrients.

This thesis is focused on the two inner layers, the endothelium and the vascular smooth muscle.

Fig 5 The vascular wall
Endothelial cells
Endothelial cells (EC) lining the lumen of all the vasculature serve as an interface between circulating blood and vascular smooth muscle cells (73). But the endothelium is not to be considered as a barrier only, it has a critical role in controlling other vascular functions. The endothelium is also an endocrine organ, with autocrine and paracrine functions. By the production and secretion of several factors and signalling molecules the endothelium has an impact on the surrounding cells in regulating vasodilatation, growth, inflammation/adhesion and haemostasis. Impaired integrity and function of the endothelium is an important factor in the development of vascular complications, if damaged adhesion for immune cells and platelets will occur (74, 75).

Vascular smooth muscle cells
Vascular smooth muscle cells form the media of the arterial wall and are found throughout the vascular tree and occur as pericytes in the capillaries. The primary function of mature differentiated VSMCs is regulation of blood vessel lumen diameter, vasoconstriction and vasodilation, thereby regulating blood flow and blood pressure. SMC are the major producers of extracellular matrix. During blood vessel repair, in vascular disease and when cultured in vitro, VSMCs dedifferentiate into a synthetic phenotype (76). The synthetic phenotype compared to the contractile phenotype is characterized by increased synthetic capacity for extracellular matrix, proteases and cytokines, increased proliferation and migration, while expression of proteins required for contraction is decreased (77).

Pericytes are important cellular constituents of the capillaries and post-capillary venules and are located abluminal to the endothelial cells and luminal to parenchymal cells. Pericytes are blood flow regulators in the microvasculature (reviewed in 78).

Atherosclerosis
Atherosclerosis, is a common pathological process that leads to cardiovascular disease, and is characterised by formation of atherosclerotic plaques, built up by foam cells, immune cells, endothelial cells, smooth muscle cells, extracellular matrix and a lipid-rich core (79). Foam cells are the main component and develop when monocyte-derived macrophages or smooth muscle cells within the arterial wall take up oxidised LDL via scavenger receptors. In addition, smooth muscle cell proliferation and migration from the vessel wall into the intima are involved in the atherosclerotic process (77). The atheroma, ie the plaque, may cause ischaemia due to narrowing of the vessel or it may rupture and cause thrombotic occlusion (80).
Diabetic macrovascular complications

The structure of the vessel wall in diabetic patients is changed and people with diabetes have a considerably higher risk for cardiovascular events than those without (77). The susceptibility to acute coronary syndrome, stroke and lower limb amputation is attributable to an increased incidence of atherothrombotic disease (3). Not only do diabetic patients have a high incidence of cardiovascular disease, they also have a worse outcome compared with non-diabetic patients, regardless of their risk factors. Diabetes itself is an independent risk factor for cardiovascular disease and the risk is further aggravated by hypertension, high cholesterol and smoking (81). There is an association between glycaemic control and diabetic macrovascular disease, and recent evidence suggests that good glycaemic control can reduce the risk for macrovascular events in both type 1 and type 2 diabetes (82, 83). However too strict glycaemic control may be dangerous (84). No single mechanism yet explains the increased risk for cardiovascular disease in diabetes.

Diabetic microvascular complications

Diabetic microangiopathy is characterised by thickening of the capillary basement membrane and in later stages by loss of microvessels (3). Diabetic microangiopathy causes severe clinical problems when affecting the eye, diabetic retinopathy, and the kidney, diabetic nephropathy, and is probably involved in diabetic neuropathy. The development of microangiopathy is closely associated with glycaemic control, and tight glycaemic control can prevent or reduce microangiopathy (85). Besides glycaemic control, other factors such as genetic susceptibility are probably of importance. Of special interest is that puberty seems to promote diabetic microangiopathy, indicating that growth factors may be involved, as GH levels and subsequently IGF-I levels have their peak at puberty (30, 86, 87).

IGF system and vascular complications

IGF-I has a dual role in cardiovascular disease, acting as a vascular protective factor, but also aggravating the atherosclerotic process. IGF-I is probably protective by stimulating endothelial nitric oxide production which has both potent vasodilatory and antiatherosclerotic properties (88). On the other hand IGF-I may contribute to the atherosclerosis process acting as a chemoattractant and stimulating VSMC proliferation (89, 90).

There is a lot of data describing vasculoprotective actions of IGF-I; low IGF-I concentrations have been associated with ischaemic heart disease (7), myocardial infarction (5) angina pectoris (6) and ischaemic stroke (91). A high free IGF-I level in the fasting state has been associated with a decreased prevalence of atherosclerotic plaques and coronary artery disease (92). IGF-I levels
have also been implicated to inversely correlate with risk of heart failure in elderly people (93). Experimental models shows that IGF-I protect cultured cardiomyocytes from apoptosis (94). Low tissue IGF-I levels and reduced IGF-I receptor expression have been found in atherosclerotic plaques (95, 96). Reduced binding of IGF-I, compared to normal, due to reduced IGF-IR expression and increased expression and secretion of IGFBPs has also been shown in plaque-derived smooth muscle cells (96). In in vitro experiments on rat aorta balloon injury have been shown to increase IGF-I production, and reduce IGF-IR expression, suggesting IGF-I to have a role in re-stenosis following angioplasty (97).

**IGF system and vascular cells**

Both IR and IGF-IR have been demonstrated in human umbilical cord vein endothelial cells, human micro- and macrovascular endothelial cells, and vascular smooth muscle cells, IGF-I receptors being much more abundant than insulin receptors (24, 98-101). Data on distribution of IR isoforms in vascular cells is sparse, but we have seen a high level of IR-A compared to IR-B in endothelial cells from the coronary artery (99).

Effects of IGF-I on VSMCs, have been well documented (102, 103). Both in vitro and in vivo studies have demonstrated that IGF-I is a stimulator of SMC proliferation. IGF-I has also been shown to have an antia apoptotic effect at low concentrations in VSMC (104). Whether or not insulin in physiological concentrations has an effect on vascular smooth muscle is controversial. Many studies regarding insulin effect on endothelial cells in vitro have been performed using very high insulin concentrations (≥10⁻⁸M) (101, 105, 106; 107). In vitro the effects of insulin on muscular arteries or cultured vascular smooth muscle cells are small and only occur at high supraphysiological concentrations (106, 108-110).

Studies of insulin and IGF-I action on endothelial cells performed using physiological concentrations are few. However, insulin at physiological levels has been shown to activate endothelial NO synthase in bovine aortic endothelial cells(111), and insulin and IGF-I at high levels have been shown to stimulate NO production in HUVEC (101).
AIMS OF THE THESIS

The overall aim of this thesis was to study mechanisms behind insulin resistance and IGF-I sensitivity and the possible impact of hybrid IR/IGF-IR in vascular cells.

Specific aims of the thesis:

- To examine insulin and IGF-I action on IR, IGF-IR, and hybrid IR/IGF-IR in vascular smooth muscle cells

- To study activation and signal transduction of IR, IGF-IR, and hybrid IR/IGF-IR by insulin and IGF-I in human microvascular endothelial cells

- To investigate the mechanism of insulin resistance in vascular smooth muscle cells by studying activation and signal transduction of IR, IGF-IR and hybrid IR/IGF-IR by insulin, IGF-I and IGF-II

- To investigate whether or not the higher gene expression of IGF-IR compared to IR in cultured vascular cells is also true in vivo.
LIST OF METHODS

The experimental procedures performed in the papers included in this thesis are listed below. For more information on the materials and methods, please find them described in detail in the respective paper.

**Paper I (rVSMCS)**
In Paper I studies on IGF-IR and IR action were performed on cultured vascular smooth muscle cells isolated from rat thoracic aorta using collagenase, which were characterised by immunostaining of smooth muscle α-actin. The studies on the cultured macrovascular smooth muscle cells included receptor abundance estimated by radioactive ligand binding. Receptor phosphorylation experiments were performed with insulin or IGF-I at physiological and supraphysiological concentrations followed by cell lysis. Detection of receptor co-precipitation and phosphorylation of receptors was performed using immunoprecipitation (IP), sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot. To control for non-specific antibody binding, of receptor antibodies used for immunoprecipitation, reduction of receptors into receptor halves prior to IP was performed with DTT treatment. Resulting biological effects were studied by analysis of proliferation using [6-3H]-thymidine incorporation assays, and metabolism using accumulation of D-[U-14C]-glucose.

**Papers II and III (HMVEC & HAMSC)**
Essentially the same analysis techniques were used in Papers II and III, performed on cultured human dermal microvascular endothelial (HMVEC) and human aortic smooth muscle cells (HASMC) respectively. These included real-time RT-PCR analysis of relative receptor mRNA abundance for IGF-IR, IR and IR isoforms. In Paper III, IGF-IR and IR expression was studied at protein level as well, using ELISA technique. IP, SDS-PAGE and Western blot analysis were used to detect phosphorylation of receptors and intracellular substrates, and resulting biological effects were studied by [6-3H]-thymidine incorporation and D-[U-14C]-glucose accumulation.

**Paper IV (LIMA)**
In Paper IV, using RT-PCR, we investigated the mRNA expression of IGF-IR compared to IR in human left internal mammary artery (LIMA).
RESULTS AND DISCUSSION

Due to the interaction of insulin, IGF-I and IGF-II at the receptor level, and common downstream signalling (112), it is important to perform studies regarding the physiological role of IGFs and insulin at physiologically relevant concentrations, otherwise the possible interaction of the polypeptides might give false results. Many in vitro studies describing insulin effects have been performed using high concentrations of insulin which may signal via the IGF-I receptor (101, 105, 106).

Insulin and IGF-I show different responses in different situations depending on receptor distribution, tissue type and cell type. Furthermore, micro- and macrovascular cells from different parts of the vascular tree may have different responses to IGF-I and insulin. We therefore studied insulin and IGF-I action in cells from different species and/or vascular beds.

Starting with IGF-IR and IR abundance studies, and dose-response phosphorylation from physiological to supraphysiological concentrations of ligands (10^{-7}-10^{-10} M), we have followed receptor phosphorylation downstream to critical intracellular nodes, and investigated resulting biological effects in macrovascular aortic cells from rat (Paper I) and human (Paper III), and microvascular dermal endothelial cells (Paper II). For an in vivo perspective, analysis of the relative IGF-IR:IR abundance in human mammary artery was performed (Paper IV).

**Low specific binding by insulin compared to IGF-I in rat vascular smooth muscle cells**

In rat macrovascular smooth muscle cells (Paper I) the densities of IGF-IR and IR were estimated by competitive radioactive ligand binding studies. Specific binding of both [^{125}I]-IGF-I and [^{35}S]-insulin could be demonstrated, and the specific binding of [^{125}I]-IGF-I (0.79%) was four-fold higher than that of [^{35}S]-insulin (0.20%), indicating a higher proportion of IGF-IR than IR, which in turn indicates a more important role for IGF-I than insulin (Fig 1 in Paper I). This is in agreement with previous results on IGF-I and insulin binding in rVSMC (20). There is, however, a possibility that binding of IGF-I to hybrid R or to IGF-BP secreted by the VSMC could have interfered with our results. However, in the earlier ligand-binding study on rVSMC by Bornfeldt et al, the binding of [^{125}I]-IGF-I could be fully displaced by insulin, ruling out the possibility that IGFBP was involved. This does not exclude the possibility of [^{125}I]-IGF-I binding to hybrid IR/IGF-IR, since the displacement by insulin occurred at a 100-fold higher concentration, but since IGF-I have high affinity for hybrid IR/IGF-IR this would still support an important role for IGF-I in vascular smooth muscle cells (20; 113).
Considerably higher gene expression of IGF-IR compared to IR in human aortic smooth muscle cells

In cultured macrovascular smooth muscle cells from human aorta (Paper III) the relative IGF-IR abundance to IR was approximately 5 times more abundant, both regarding mRNA expression and protein levels, $5.3 \pm 1.3$ (p<0.001) and $5.5 \pm 1.2$ (p<0.001) respectively (Fig 2 in Paper III). This is in agreement with previous studies on cultured smooth muscle cells from human coronary artery, where our group demonstrated an eight times higher abundance of IGF-IR mRNA compared to IR mRNA expression (98). In a previous paper on microvascular endothelial cells by Chisalita & Arnqvist 2004, human microvascular endothelial cells (HMVEC) were shown to have five times more IGF-IR mRNA than IR. Also, in the same study the specific binding of insulin in HMVEC was shown to be several times lower than the specific binding of IGF-I indicating a lower number of homodimeric IR compared to IGF-IR or insulin/IGF-I hybrid receptors (24).

High IRA to IRB gene expression in vascular smooth muscle and endothelial cells

In macrovascular human smooth muscle cells (Paper III), IR-A mRNA was found to be thirteen times more abundant than IR-B (p< 0.001), which to our knowledge has not previously been reported in vascular smooth muscle cells. Regarding microvascular endothelial cells, Paper II, the IR isoform A (IR-A) mRNA abundance relative to IR-B mRNA expression was 7 times higher ($7.6 \pm 1.2$) (p < 0.01). The domination of the IRA isoform in HMVEC, is in agreement with earlier results on macrovascular endothelial cells from coronary artery (99).

Co-precipitation of insulin and IGF-I receptor β-subunits demonstrating hybrid receptors in vascular smooth muscle and endothelial cells

In Papers I-III, cultured rVSMC, HMVEC and HASMC were analysed for the presence of receptor β-subunits using IP and SDS-PAGE. (See Fig 2 in Paper I, Fig 2 in Paper II and Fig 3 in Paper III). After IP and Western blot with IGF-IR β -subunit specific antibodies we could detect a band at the level of the 97 kDa molecular marker, corresponding to the molecular weight of the IGF-IR β-subunit. When the membrane was stripped and probed with the IR β –subunit-specific antibody, a band at a clearly lower position could be detected, corresponding to the IR β-subunit (95 kDa). After IP with an IR β –subunit-specific antibody, bands at the same levels were detected with the specific IR and IGF-IR antibodies respectively. To control for non-specific binding of the antibodies used for immunoprecipitation (IP), reduction of receptors into receptor halves prior to IP was performed using DTT treatment (Papers I-II). The presence of hybrid receptors is in agreement with previous findings in vascular cells, both in vascular smooth muscle and endothelial cells (98-100).
Phosphorylation of receptor β-subunits resulting in double bands in rat macrovascular smooth muscle cells

In rVSMC, at a high concentration, $10^{-8}$ M, both IGF-I and insulin stimulated the phosphorylation of IGF-IRβ and IRβ. However, at physiological concentrations, $10^{-9}$-$10^{-10}$ M, IGF-I dose-dependently enhanced the tyrosine phosphorylation of IRβ and IGF-IRβ, but insulin only stimulated its own cognate IRβ subunit, see Figure 4 in Paper I. The ability of IGF-I to phosphorylate IR even at a low concentration, together with the fact that immunoprecipitation of IRβ pulled down the IGF-IRβ and vice versa, indicates the presence of hybrid IR/IGF-IR in rVSMC. The activation by IGF-I of co-precipitated IRβ and IGF-IRβ subunits, visible as double bands on the blot, suggests that IGF-I activates hybrid receptors as well. Hence, in Paper I our results on rVSMC show IGF-I but not insulin to be able to phosphorylate the other receptor at low concentrations ($10^{-9}$-$10^{-8}$ M), indicating activation at physiologically relevant concentrations (114). This activation of IR, even at a low IGF-I concentration $10^{-9}$ M, indicates that the activated IR β-subunits had been sequestered into hybrid IR/IGF-IR which can be activated by IGF-I even at low concentrations.

IGF-I activates receptors and downstream signalling in microvascular endothelial cells

In a previous paper on HMVEC (24), IR and IGF-IR phosphorylation in HMVEC were studied at high supraphysiological concentrations ($10^{-6}$ and $10^{-8}$ M) only, whereas in Paper II we studied receptor activation of IR and IGF-IR at low physiological concentrations of insulin and IGF-I also. In Paper II significant phosphorylation of the IGF-IR β-subunit in HMVEC compared to baseline was caused by IGF-I stimulation at concentrations $10^{-9}$-$10^{-8}$ M, which also stimulated significant phosphorylation of the IR β-subunit and IRS-1 (see Fig. 3-4 in Paper II). IGF-I stimulation at concentrations $10^{-8}$-$10^{-7}$ M phosphorylated Akt (Fig. 4B in Paper II). Insulin on the other hand did not manage to activate either of the receptors or IRS-1 at the concentrations used ($10^{-9}$-$10^{-8}$ M, as seen in Figure 4A in Paper II). However, at the high supraphysiological concentration, $10^{-7}$ M, Akt was significantly phosphorylated by insulin. In HMVEC, neither insulin nor IGF-I within the range of $10^{-10}$-$10^{-7}$ M elicited significant ERK 1/2 phosphorylation, as shown in Fig. 4C in Paper II.

Activation of receptors and downstream signalling in human macrovascular smooth muscle cells

Similarly, in HAMSC, insulin $10^{-10}$-$10^{-8}$ M did not significantly phosphorylate its cognate receptor, IGF-IRβ, IRS-1, Akt nor Erk 1/2 (Figure 4 – 5 in Paper III). In HAMSC (Paper III) we also found IGF-I to phosphorylate its own receptor at $10^{-9}$-$10^{-8}$ M and the IRβ at $10^{-8}$ M. We also studied the effects of IGF-II, which at $10^{-8}$ M phosphorylated the IGF-IRβ, but did not propagate any intracellular signalling. As regards intracellular signalling in HASMC, IGF-I significantly phosphorylated IRS-1 at
Insulin resistance and IGF-I sensitivity in vascular cells
- impact of hybrid receptors

10^{-8} M and tended to phosphorylate IRS-1 at 10^{-9} M (p = 0.12). IGF-I also significantly phosphorylated Erk 1/2 at 10^{-9}-10^{-8} M, and although not significantly so, IGF-I tended to stimulate Akt at 10^{-8} M (p = 0.15).

**Biological effects seen at physiological concentrations of IGF-I, but not insulin, in rat macrovascular smooth muscle cells**

The effect of ligands on glucose metabolism in rat vascular smooth muscle cells (Paper I) was analysed as accumulation of D-[U-14C]-glucose. Glucose accumulation was markedly elevated in cells stimulated with IGF-I 10^{-10}-10^{-7} M, and the maximum accumulation level was reached at 10^{-9} M. Insulin stimulation, however, did not reach maximum effect until at 10^{-7} M (Fig. 6 in Paper I).

Regarding the effects on glucose uptake in endothelial and vascular smooth muscle cells, the major glucose transporter protein (GLUT) is GLUT-1 (115). This is a house-keeping GLUT primarily responsible for basal, i.e. insulin-independent, glucose uptake (116).

**Insulin does not increase cell proliferation in microvascular endothelial cells**

In Paper II, HMVEC, [6-3H] thymidine incorporation was significantly increased from baseline in cells stimulated with IGF-I 10^{-8}-10^{-6} M, maximum levels being reached at 10^{-8} M, whereas insulin stimulation did not show any significant increase from baseline, although an increase in effect could be seen at 10^{-8} M see Fig. 5 in Paper II. Data are expressed as per cent above control (unstimulated) cell radioactivity.

**Biological effects at physiological concentrations of IGF-I and IGF-II, but not insulin, in rat aortic smooth muscle cells**

In Paper I, rVSMC cell proliferation measured as [6-3H]-thymidine incorporation into DNA showed a pronounced effect by IGF-I even at a low concentration, 10^{-10} M, reaching a maximum effect at 10^{-9} M. Stimulation with insulin showed a measurable effect only at high concentrations, 10^{-8}-10^{-7} M (Fig 7 in Paper I).

In Paper III, HASMC, incorporation of [3H]-thymidine was stimulated by 10^{-8}-10^{-7} M IGF-I (p = 0.02 and p = 0.006) and IGF-II (p = 0.001 and p ≤ 0.001), whereas insulin had no significant effect (Fig 7A in Paper III). Glucose accumulation was significantly stimulated by concentrations of 10^{-8}-10^{-7} M IGF-I (p=0.03 and p=0.001) and IGF-II (p<0.001, p<0.001), and also by insulin at the highest concentration of 10^{-7} M (p=0.001) (Fig 7B in Paper III).
**High relative IGF-IR : IR expression in LIMA - also true in vivo**

Since cells can dedifferentiate during culture the characteristics of cultured cells may be altered compared to the in vivo situation. We investigate whether or not the considerably higher gene expression of IGF-IR compared to IR was also true in vivo. The gene expression of IGF-IR in all samples was 36 times higher (35.6 (22.1-54.6)) than IR (n=12, p < 0.0001) (Fig 1 in Paper IV).

Although one must take into consideration that the LIMA samples were very few and all tissue was collected from CAD patients, the higher expression of IGF-IR seems also to be true in vivo.
GENERAL DISCUSSION

Insulin resistance in vascular cells

Our in vitro data suggest that insulin has no or little effect on the human vascular cells studied, smooth muscle cells and endothelial cells. In Paper I and III and a previous paper regarding smooth muscle cells from coronary artery Chisalita et al 2005 (98) we found no effects on growth or metabolism. In Paper II and our previous report on microvascular endothelial cells Chisalita and Arnqvist 2004 (24) low concentrations of IGF-I but not insulin were found to stimulate biological effects. The lack of insulin effect on DNA-synthesis in HMVEC is in agreement with our previous observations (24) together with several other reports on microvascular cells (117; 118) suggesting that human microvascular endothelial cells are insulin resistant. In contrast to our results in vitro, clinical studies in vivo have demonstrated obvious circulatory effects of insulin (71). However the mechanism behind this direct action on vascular cells is not elucidated.

Importance of the IGF system in the vascular cells

As discussed above regarding the IGF-I action, vascular smooth muscle cells and endothelial cells were shown to be sensitive to IGF-I. Overall our results on receptor activation, downstream signalling and biological effects show vascular cells to be sensitive to IGF-I but not to insulin. This is agreement with earlier studies from our group on cultured endothelial and vascular smooth muscle cells from different vascular beds (24, 98-100).

In this thesis the cultured vascular cells were characterised by a preponderance of IGF-IR and few insulin receptors, which is of importance for IGF-I sensitivity. Studies on freshly isolated HUVEC cells have shown fresh and cultured endothelial cells to have the same characteristics regarding the ratio of IGF-IR to IR gene expression (100). Supported by our similar results on human arterial tissue, this implies that our results on cultured cells could be applied to the in vivo situation. The function of IGF-I in intact vascular tissue has been studied in vivo, where the infusion of IGF-I in the human forearm model has been shown to increase blood flow (119). Considering the fact that patients with diabetes have dysregulation of the IGF system with low circulating IGF-I levels and changes in the IGF-binding proteins (33) it is possible that these changes might affect the function of vascular cells.
The relevance of physiological ligand concentration due to cross-reactivity

The high homology between insulin and IGFs and their cross-over activation of IR, IGF-IR and hybrid IR/IGF-IR can confound interpretation of results from studies on insulin and IGFs action where high ligand concentrations have been used. Many studies regarding insulin effect on endothelial cells in vitro have been performed using very high insulin concentrations (≥10^{-8} M) (101, 105, 106; 107). Due to the possible interaction of insulin with IGF-I receptors at high concentrations it is therefore possible that the effects observed in those studies have been mediated by IGF-I receptors.

High expression of IRA compared to IRB

Concerning the binding affinity of IRA and IRB, it is of interest to note that IRA, which has a higher binding affinity for IGF-I and IGF-II than IRB (56), is more abundantly expressed than IR-B in vascular cells. Interestingly, the classical insulin-responsive tissues express mainly IR-B compared to IR-A (57). In our experiments we found no activation of insulin receptors by either insulin or IGF-II, probably due to the low number of IR holoreceptors.

A physiological role for IGF-II?

IGF-II is the dominating IGF in the circulation of adult human and is present at a very high level, although how much is free and bioactive is debated (9, 10; 16). We observed IGF-II mediated activation of receptors and biological effects at 10^{-8} M which could suggest that IGF-II has physiological role in human vascular smooth muscle.

Impact of hybrid receptors

There are several mechanisms proposed to be involved in the pathophysiology of insulin resistance; the influence of increased levels of free fatty acids and pro-inflammatory molecules, impaired muscle glycogen synthesis, defects in glucose uptake and insulin intracellular signalling, and more (reviewed in 67). Also, the expression of hybrid IR/IGF-IR in classical insulin target tissues have been correlated with insulin sensitivity in vivo (69, 70). In our studies on vascular cells, insulin at physiological concentrations had difficulties activating its own receptor, and could not induce intracellular signalling nor propagate biological effects. This insulin insensitivity is probably due to the receptor distribution in vascular cells, including hybrid IR/IGF-IR. Since the relative stoichiometric abundance of IGF-IR and IR determines the abundance of hybrid receptors, most IR will be incorporated into hybrid receptors resulting in few insulin holoreceptors and decreasing insulin sensitivity, as exemplified in Figure 8.
The importance of this sequestration of IRs into hybrid IR/IGF-IR for insulin action has been shown in several studies. Down-regulation of IGF-IR was shown to increase insulin sensitivity in both VSMC (40), osteoblasts (41) and breast cancer cells (42).

Taken together, we believe hybrid IR/IGF-IR might be a mechanism regulating insulin resistance at the receptor level in vascular cells.

*Proposed model for insulin resistance in vascular cells*
SUMMARY

- In rat vascular smooth muscle cells, human microvascular endothelial cells and human aortic smooth muscle cells the presence of hybrid IR/IGF-IR was demonstrated by co-precipitation of the IR and IGF-IR β-subunits.

- In rat vascular smooth muscle cells, insulin at physiological concentrations (≤10^{-9} M) stimulated tyrosine phosphorylation of the IR but did not affect either glucose metabolism or DNA synthesis, whereas IGF-I at physiological concentrations stimulated phosphorylation of both IGF-IR and IR and elicited a biological effect.

- In human microvascular endothelial cells, IGF-I, in contrast to insulin, stimulated receptor phosphorylation followed by downstream signalling and increased DNA-synthesis at physiologically relevant concentrations.

- In human microvascular endothelial cells and human aortic smooth muscle cells, the level of IR-A was found to be considerably higher than IR-B.

- In human aortic smooth muscle cells, the expression of IGF-IR was shown to be higher than IR at both mRNA level and protein level.

- In human aortic smooth muscle cells, IGF I and IGF-II, but not insulin, elicit receptor phosphorylation and subsequent biological effects.

- In human arterial tissue the gene expression of IGF-IR was found to be considerably higher than IR in vivo.
CONCLUSIONS

We conclude that:

1. The relative abundance of IGF-IR is considerably higher than IR in vascular cells in vitro and in vivo

2. In addition to IR and IGF-IR, hybrid IR/IGF-IR are present in vascular cells

3. IR activation at physiological concentrations (≤10-9 M) does not propagate downstream signalling and biological effects in endothelial and vascular smooth muscle cells.

4. Low concentrations of IGF-I activate IGF-IR, as well as IR due to the presence of hybrid IR/IGF-IR, and propagate downstream signalling and biological effects in endothelial and vascular smooth muscle cells.

5. The significant biological effects mediated by IGF-II suggests a role for IGF-II in vascular smooth muscle cells.

Taken together, the papers included in this thesis provide important new insight on how IGFs and insulin act in the vasculature. The preponderance of IGF-IR relative to IR in addition to sequestration of IR into hybrid IR/IGF-IR contributes to an insulin resistance located at the receptor level in endothelial and vascular smooth muscle cells. Hence, our results suggest that IGFs rather than insulin have an impact on vascular function.
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* * *

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SVENSK SAMMANFATTNING


Den här avhandlingen baseras på fyra arbeten som undersökt förekomst av IR och IGF-IR i odlade endotelceller och glatta muskelceller från kärl, samt i vävnad från human vänster inre bröstartär (LIMA). I odlade endotelceller och glatta muskelceller från kärl har vi undersökt fosforlysering av IR och IGF-IR, samt IR- och IGF-IR medierade svar, dvs vidareering nedströms och biologiska effekter, till följd av stimulering med insulin, IGF-I och IGF-II vid fysiologiska och suprafysiologiska koncentrationer. Vi har också undersökt förekomst av hybrid IR/IGF-IR i dessa celltyper. För att relatera våra in vitro försök till situationen in vivo, undersökte vi även det relativa genuttrycket av IGF-IR jämfört med IR i LIMA.

Vi drar följande slutsatser: 1) den relativa förekomsten av IGF-IR är betydligt högre än IR i kärlceller in vitro och in vivo; 2) förutom IR och IGF-IR förekommer i celler från kärlvägg även hybrid IR/IGF-IR; 3) IR aktiverad vid fysiologiska koncentrationer (≤10-9 M) propagera inte vidare intracellulär signalering eller biologiska effekter, i endotelceller och glatta muskelceller från kärl; 4) låga koncentrationer av IGF-I aktivera IGF-IR, och även IR tack vare förekomsten av hybrid IR/IGF-IR, samt propagera vidare intracellulär signalering och biologiska effekter i endotelceller och glatta muskelceller från kärl; och 5) de biologiska effekterna till följd av IGF-II-stimulering föreslår en roll för IGF-II i glatta muskelceller från kärl.

Arbetena inkluderade i den här avhandlingen bidrar med nya insikter i hur IGFs och insulin verkar i kärlvägg. Det övervägande antalet IGF-IR relativt IR samt inkorporeringen av IR in i hybrid IR/IGF-IR orsakar en insulinresistens lokalisera på receptornivå i endotelceller och glatta muskelceller från kärl. Sammantaget talar våra resultat för att IGFs snarare än insulin är av vikt för kärlväggens funktion.
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